

Computational Toxicology and Endocrine Disruptors: Use of Systems Biology in Hazard Identification and Risk Assessment

The U.S. Environmental Protection Agency (EPA) is interested in the application of novel technologies that are derived from computational chemistry, molecular biology, and systems biology and used in toxicological risk assessment.

In assessing risk associated with exposure to a chemical or other environmental stressor, a number of scientific uncertainties exist along a "source-to-adverse-outcome" continuum, beginning with the presence of the chemical in the environment, the uptake and distribution of the chemical in the organism or environment, the presence of the active chemical at a systemic target site, and the series of biological events that lead to the manifestation of an adverse outcome that can be used for risk assessment. The EPA Office of Research and Development (ORD) Human Health Research Strategy (available online at <http://www.epa.gov/sab/pdf/hhrs.pdf>) describes these scientific uncertainties and some of the multidisciplinary approaches that are needed to build linkages between exposure, dose, and effects.

The ORD has initiated a new research program called Computational Toxicology (see http://www.epa.gov/nheerl/comptoxframework/comptoxframeworkfinaldraft7_17_03.pdf) that will use emerging technologies to improve risk assessment and reduce uncertainties in this source-to-adverse-outcome continuum. This initiative, as well as work being conducted by other institutions and organizations, will provide a wealth of information on effects of toxicants at multiple levels of biological organization by using genomic, proteomic, and metabonomic techniques. In order to be most useful, this information must be integrated into a coherent picture.

One of the strategic objectives of the Computational Toxicology initiative is to develop improved linkages across the continuum, including the areas of chemical transformation and metabolism, better diagnostic/prognostic molecular markers, improved dose metrics, characterization of toxicity pathways, metabonomics, systems biology approaches, modeling frameworks, and uncertainty analysis. This solicitation for research proposals is focused on development of systems biology-based models for key components of adverse health outcomes induced by environmental contaminants.

Systems biology uses computational methods to reconstruct an integrated physiologic and biochemical model of an organism's or cell's biology. The approach is similar to developing a wiring diagram for a complicated electrical system or an engineering diagram, such as one that shows the function and interaction of different parts of an automobile. In this regard, systems biology is targeted at studying how normal biological processes are governed, and how alterations can lead to diseases or other unwanted outcomes. Understanding the functions of a normal cell or organism is key to understanding how toxicants can exert effects.

A systems biology approach will enable the integration of disparate data developed by biologists, computer scientists, chemists, engineers, mathematicians, and physicists to construct models of organism function and response to toxic

insult. For any model, a choice must be made about scale and level of detail. In this case, models useful to the EPA most likely will be built from individual subcomponents assembled into a larger system. Once these models are developed, then hypotheses can be developed and tested through virtual simulations prior to designing targeted experiments to validate and inform the models. An integral part of the Computational Toxicology initiative will be the use of relevant model organisms to expand our understanding of the regulation of biological processes and how toxicants can perturb these processes, with the goal of identifying the key mechanistic events for improved risk prediction.

The brain-pituitary-gonadal and brain-pituitary-thyroid axes represent two complex endocrine pathways important for reproduction and survival. Growing evidence exists that these two axes are targets for a variety of environmental toxicants. For example, disruption of thyroid homeostasis may occur in response to environmental compounds that affect thyroid synthesis (iodine uptake or peroxidase inhibitors), transport (disrupting binding to thyroglobulin), excretion (phase I and phase II liver enzymes), uptake, and utilization (deiodinate). Of equal complexity, the hypothalamic-pituitary-gonadal axis has been shown in both sexes to be sensitive to disruption by environmental agents that affect gonadotropin secretion, steroidogenesis, receptor binding, or signal transduction.

Of particular interest is a better characterization of the mechanisms involved in toxicant-induced alterations in the hypothalamic control of gonadotropin-releasing hormone (GnRH) and thyrotropin-releasing hormone (TRH). Thus, there is a need for the development of protocols and techniques that provide quantitative measures of change within the GnRH and TRH neurons directly, as well as those that quantify changes in the neuronal systems that regulate these cells.

Integrated studies need to be developed that merge the rich basic literature detailing the molecular and biochemical mechanisms involved in the normal functioning of the gonadal and thyroid axes with studies evaluating and characterizing the adverse effects of environmental chemicals on these systems. These efforts should be facilitated by the application of a systems biology approach that could eventually lead to the development of predictive models. Furthermore, due to the complex nature of the regulatory processes of these neuroendocrine axes, incorporation of new technologies is needed so that multiple molecular and biochemical parameters can be evaluated to determine how classes of environmental toxicants affect the systems' homeostasis.

It is envisioned that the development and application of these techniques will assist in the identification and interpretation of changes in the synthesis of reproductive and/or thyroid hormones, hormonal release/clearance, steroid and thyroid receptor regulation (e.g., synthesis, binding, activation), and the hypothalamic peptides and neurotransmitters involved in the regulation of GnRH and TRH.

The EPA, through its Science to Achieve Results (STAR) program, is seeking applications proposing innovative approaches for incorporating

computational methods into hazard identification and risk assessment. These proposals should incorporate a systems biology approach with chemical effects assessment using traditional and novel techniques, which should include genomics, proteomics, and metabonomics, as well as formal mathematical descriptions of the relevant physiology and pharmacodynamic aspects. The proposal should focus on one or both of the following investigational areas:

- 1) Development of integrative, quantitative models of the function of the hypothalamic-pituitary-gonadal or hypothalamic-pituitary-thyroid axis with emphasis on the descriptions of the normal physiological processes and mechanisms of perturbation following exposure to xenobiotics (e.g., endocrine-disrupting chemicals) in rats or a commonly used small fish toxicology model (e.g., fathead minnow, medaka, zebrafish).

- 2) Cross-species extrapolation of integrative, quantitative models of the perturbed hypothalamic-pituitary-gonadal or hypothalamic-pituitary-thyroid axis following exposure to xenobiotics from rats to humans or a commonly used small fish toxicology model (e.g., fathead minnow, medaka, zebrafish) to other vertebrates (i.e., within the same class or across classes). The cross-species extrapolation should be based on models as described in item 1, above. Proposals should demonstrate the existence of these models and their validity.

The tools of modern molecular biology, including genomics, proteomics, and/or metabonomics, should help to elucidate the interrelationships among the key components of the endocrine system under study, how these hormonal pathways maintain homeostasis, and, ultimately, how these systems react in response to toxic insults. It would be beneficial if the dose metric put into the model were derived from a similar physiologically based toxicokinetic model. The models may initially be developed for one sex of the adult organism, but should be adaptable to either sex and to other life stages (particularly the developing organism). Development, testing, and validation of these models will require collaboration among molecular biologists, endocrinologists, and mathematical biologists.

It is anticipated that a total of approximately \$2.4 million will be awarded, depending on the availability of funds. Approximately 3–5 awards will be made under this request for applications. The projected award per grant is \$150,000–250,000 per year total costs, for up to 3 years. Requests for amounts in excess of a total of \$750,000, including direct and indirect costs, will not be considered.

A set of special instructions on how applicants should apply for a National Center for Environmental Research grant along with the forms required for application are found on the center website at <http://es.epa.gov/ncer/rfa/forms/>, Standard Instructions for Submitting a STAR Application. The deadline for receipt of applications is 21 January 2004. Complete information on this announcement is located at http://es.epa.gov/ncer/rfa/current/2003_comptox.html.

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Environmental Statistics Research: Novel Analyses of Human Exposure Related Data

Human exposure to toxic substances and the resulting health risks are a function of the number and concentrations of substances in the environment, their health potency, and the frequency and duration of human contact with these substances. The assessment of these exposures and risks is an inherently uncertain activity. Every step calls upon the analyst to make sense of uncertain and variable information. In addition, aggregate exposure and cumulative risk have become critical features of environmental decision making.

Ultimately, the challenge of interpretation falls to the decision makers for whom the assessments are performed. Because exposure assessments are relatively young applications of statistical tools and scientific principles, it is critical that methodologies be developed for addressing, quantifying, and presenting the uncertainty and variability in the models, the model inputs, and the outputs upon which the field relies. This type of research will benefit a wide range of scientists and environmental managers as they take into consideration multichemical, multipathway exposures when setting environmental standards as mandated.

The growing concern among policy makers and scientists that the uncertainty currently associated with risk assessment is unacceptable has led to an increased interest in collecting better data to assess aggregate and cumulative exposures. For example, both the Environmental Protection Agency (EPA) and the chemical industry are striving to reduce uncertainties associated with the risk assessments they use to make decisions concerning the safety of pesticides for both agricultural and residential use. Other programs addressing such diverse issues as air quality, lead-based paint in older housing, drinking water, and hazardous waste sites are also taking aggregate and cumulative exposures into consideration in their risk assessments.

In response to the needs of environmental decision makers, research efforts have led to a marked increase in the quality and amount of data available on human environmental exposures. Data collected during these studies usually consist of concentrations of multiple target chemicals measured in various environmental media such as indoor and outdoor air, tap water, soil, food, and house dust; concentrations of chemicals in blood, urine, and other body fluids and tissue; and behavioral and demographic information collected through diaries and questionnaires.

The exposure of children has also been a high priority for environmental exposure data collection activities over the last few years—recent studies have collected microenvironmental concentrations, human activity information, and biomarker data for children aged 0–12 in several regions of the United States and Canada. These data are beginning to shed light on the unique factors that influence the exposure of this vulnerable subpopulation.

Both the EPA and the American Chemistry Council (ACC) continue to conduct and support the collection of human exposure data. However, given the cost and complexity of these studies, there is rarely sufficient funding available for the

analysis of these data beyond that necessary to test the original study hypotheses. Therefore, they are also interested in supporting research to develop innovative statistical methods and models that will improve our understanding of human exposure and provide improved methods for assessing exposure and risk. By supporting such multidisciplinary and innovative research, the EPA National Center for Environmental Research (NCER) and the ACC believe that the science that underpins the exposure and risk assessment approaches used in environmental decision making can be improved. Enhancing our understanding of exposures by capitalizing on these valuable existing data resources is another important step to a better understanding of the relationships between human health and the environment, and the physical and human dimensions of environmental policies.

Earlier this year, the NCER and the ACC signed a memorandum of understanding (MOU) to utilize the complementary expertise and capabilities of the EPA and the ACC in the joint development of requests for applications (RFAs) for areas of research of mutual interest. The MOU sets forth the procedure that the NCER and the ACC will follow for the coordination, collaboration, and cooperative issuance of joint solicitations of research proposals (<http://www.epa.gov/ncer/rfa/partners/acc>).

In accordance with the public procedure established in the MOU, a *Federal Register* notice was issued on 7 May 2002 (67 FR 30680) that identified an interest in pursuing a joint request for applications in the area then called “Novel Approaches for Analysis of Human Exposure Data.” Comments were solicited at the public meeting held on 23 May 2002, and the written comment period ended on 12 June 2002. This current announcement marks the issuance of the first joint solicitation.

In this solicitation, the NCER and the ACC are requesting applications that propose novel statistical analyses of existing chemical and behavioral data to develop new exposure information, methods, or approaches that ultimately can be used in a variety of exposure measurements, models, and/or assessments. The analyses, for example, can be used to provide estimates of various exposure concentrations and exposure factors for exposure assessments. The exposure assessments often rely on point estimates or distributions obtained from limited data sets that may not be appropriate to the populations of interest for that assessment. Further, these analyses may help determine the potential bias in estimates of national exposure factors and distributions which may result from the use of local or regional sampling. These analyses can also contribute to the development of indicators or indices that can be used to evaluate the progress or effectiveness of environmental decision making on reducing levels of exposure.

The NCER and the ACC are interested in funding efforts to conduct a variety of descriptive analyses on exposure-related data. Possible areas of investigation include but are not limited to 1) novel analyses of distributions of media concentrations, exposure, and biological measurements for population subgroups by age, gender, ethnicity, socioeconomic status, urban versus rural, or other important groupings; 2) descriptive statistics (distributional

information) for exposure-related data that can be used broadly in exposure assessment and in the design of human health effects studies; 3) analyses of method sensitivity and precision—how the censoring of data below detection limits (and alternative methods to treating these data in the analyses) affects the estimation of distributions for exposure, media concentration, and biomarker measurements, and the evaluation of associations among such measurements; 4) probabilistic methods for assessing multipathway exposures and methods for linking information about contaminant source, transport, and human interactions with their environment; 5) analysis of the intra-individual variability in biological, chemical, and behavioral factors that contribute to exposure and determine the importance of this variability in estimating exposure; 6) methods for extrapolating small data sets to estimate population-level exposure and effects, and methods to expand the use of epidemiological data in risk assessment; 7) multivariate statistical and model-based methods to determine relationships between concentrations in environmental media (personal air, dust, diet) and biological samples that can characterize the resultant uncertainty in estimates of environmental exposure; 8) examination populations that are highly exposed to multiple chemicals; 9) estimation or classification of exposures based on existing environmental monitoring data or models, and comparisons with direct exposure measurements; 10) methods for representing and communicating the limits and uncertainty of environmental data, as well as approaches for characterizing and reducing uncertainty in environmental exposure and risk assessment; 11) visualization tools to assist exposure assessors in interpreting the relationships between exposure-related data sets; and 12) estimates of exposure to specific environmental toxicants in the specific populations occurring via all routes and pathways.

It is anticipated that a total of approximately \$2 million will be awarded, depending on the availability of funds. A total of approximately 5–10 awards will be made under this RFA. An award will be funded by only one of the sponsoring organizations. Awards made by the EPA will be in the form of grants. Awards made by the ACC will be in the form of research contracts following the principles of investigator independence (see <http://www.uslri.org/> for more information). The projected award per grant is \$50,000–150,000 per year total costs, for up to 3 years. Requests for amounts in excess of a total of \$450,000, including direct and indirect costs, will not be considered.

All applicants, regardless of whether they specify funding from either the EPA or the ACC, or only the EPA, are required to use the STAR application forms. The Standard Instructions for Submitting a STAR Application including the necessary forms are available on the NCER website, <http://es.epa.gov/ncer/rfa/forms/>. The deadline for receipt of applications is 14 January 2004. Complete information on this RFA is located at http://es.epa.gov/ncer/rfa/current/2003_enviro_stat.html.

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